Practical information

- Experiments and talks
  - Short 10 min intro every day before the first lab session
- Work in the lab
    - Lab coats
    - Waste
    - P2 rules (for tagged *P. aeruginosa*). If anyone is pregnant let us know
- Talks every day
- Presentations at the last day
  - Treat data/results on the way so that you “just” have to put everything together in the end
- The manual
    - Schedule for the different experiments
    - Flowchart of the different experiments
    - List of speakers
    - Laboratory manuals and protocols
    - The flow systems
    - Additional protocols
    - Accompanying papers
- Valuables
- Teams
- The Coffee machine

Working in P2 classified lab

- Only max. 3 groups inside P2 at a time due to limited work space.
- Introduction to the P2 lab is carried out by the supervisor.
- In general:
- Leave your own lab coat, course material, protocols, pencils, etc. outside the P2 lab.
- Wear the lab coats and shoe covers which are provided in the P2 lab.
- Wear protective gloves when working.
- All waste, syringes etc. go into special waste-containers (metal buckets, yellow-cap boxes).
- Wash your hands before leaving the P2 lab.

The exercises

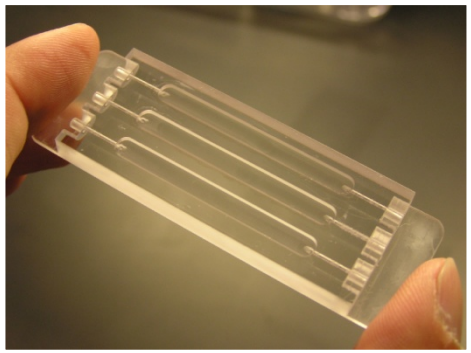
- **5 different biofilm systems will be set up:**
- System 1: Development and architecture of *E. coli* biofilms
- System 2: FISH in biofilms(*P. aeruginosa and Acinetobacter* variants)
- System 3: Differentiation, structure development and colistin tolerance in *P. aeruginosa* biofilms
- System 4: *P. aeruginosa* mutants structure development and resistance
- System 5: Free Exercise
- Characterization of *Pseudomonas aeruginosa* from chronic ear infections in dogs
- Microtiter assays of different strains in connection to system 1 and 4
- Additionally:
  - The biofilm protocol
  - The FISH protocol
  - Adhesion assay
  - Conjugation/plasmid transfer
  - MIC determinations
  - Cell sorting
  - Quorum Sensing assays

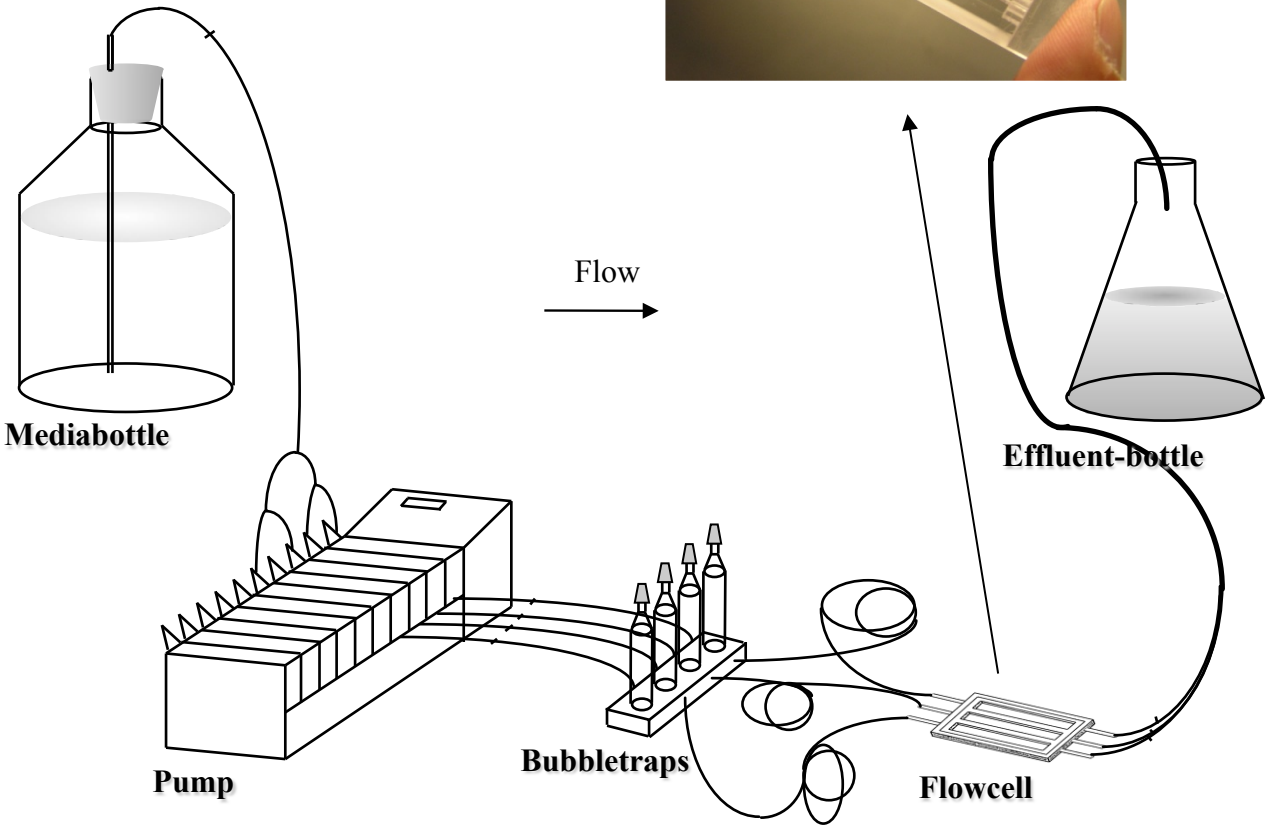
Experiments day 1

The Flow Chamber System

The standard set-up at DTU for hydro-dynamic biofilm development.

The flow-cells are mountable directly on the Confocal Microscopes for in situ investigations (Zeiss LSM510 or Leica DMRXA)





Biofilm system 1:
E. coli biofilm development, the affect of F pilus

Isogenic F plasmid derivatives:

traA: **No pilus synthesis**

traD**: No DNA transfer**

F⁺ **F*traA*** **F*traD***

Images for COMSTAT at 2 time points during the next days

Biofilm system 3:

- Impact of type IV pili-driven motility on structural biofilm formation

P. aeruginosa wild-type Yfp + *P. aeruginosa pilA* Cfp

- Tolerance-development towards Colistin in biofilms

P. aeruginosa wild-type Gfp + Colistin + Propidium Iodide